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Polynucleotide encoding a polypeptide having heparanase activity and expression of same in transduced cells

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(54) Title: POLYNUCLEOTIDE ENCODING A POLYPEPTIDE HAVING HEPARANASE ACTIVITY AND EXPRESSION OF SAME IN TRANSDUCED CELLS

(57) Abstract

A polynucleotide *(hpa)* encoding a polypeptide having heparanase activity, vectors including same, transduced cells expressing heparanase and a recombinant protein having heparanase activity.

The claims defining the invention are as follows:

1. A polynucleotide fragment comprising a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity, wherein said polypeptide shares at least 70 % homology with SEQ ID NOs:10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

2. The polynucleotide fragment of claim 1, wherein said polynucleotide sequence includes nucleotides 63-1691 of SEQ ID NO:9, or nucleotides 139-1869 of SEQ ID NO:13.

3. The polynucleotide fragment of claim 1, wherein said polynucleotide sequence includes nucleotides 63-721 of SEQ ID NO:9.

4. The polynucleotide fragment of claim 1, wherein said polynucleotide is as set forth in SEQ ID NOs:9 or 13.

5. The polynucleotide fragment of claim 1, wherein said polynucleotide sequence includes a segment of SEQ ID NOs:9 or 13, said segment encodes said polypeptide having said heparanase catalytic activity.

6. The polynucleotide fragment of claim 1, wherein said polypeptide includes an amino acid sequence as set forth in SEQ ID NOs:10 or 14.

7. The polynucleotide fragment of claim 1, wherein said polypeptide includes a segment of SEQ ID NOs:10 or 14, said segment harbors said heparanase catalytic activity.

8. The polynucleotide fragment of claim 1, wherein said polynucleotide sequence is selected from the group consisting of double stranded DNA, single stranded DNA and RNA.

9. A polynucleotide fragment comprising a polynucleotide sequence at least 70 % homologous with SEQ ID NOs:9 or 13, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin, said polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

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10. The polynucleotide fragment of claim 9, wherein said polynucleotide sequence is as set forth in SEQ ID NOs:9 or 13.

11. A vector comprising a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity, wherein said polypeptide shares at least 70 % homology with SEQ ID NOs:10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

12. The vector of claim 11, wherein said polynucleotide sequence includes nucleotides 63-1691 of SEQ ID NO:9, or nucleotides 139-1869 of SEQ ID NO:13.

13. The vector of claim 11, wherein said polynucleotide sequence includes nucleotides 63-721 of SEQ ID NO:9.

14. The vector of claim 11, wherein said polynucleotide sequence is as set forth in SEQ ID NOs:9 or 13.

15. The vector of claim 11, wherein said polynucleotide sequence includes a segment of SEQ ID NOs:9 or 13, said segment encodes said polypeptide having said heparanase catalytic activity.

16. The vector of claim 11, wherein said polypeptide includes an amino acid sequence as set forth in SEQ ID NOs:10 or 14.

17. The vector of claim 11, wherein said polypeptide includes a segment of SEQ ID NOs:10 or 14, said segment harbors said heparanase catalytic activity.

18. The vector of claim 11, wherein said polynucleotide sequence is selected from the group consisting of double stranded DNA, single stranded DNA and RNA.

19. The vector of claim 11, wherein said vector is a baculovirus vector.

20. A vector comprising a polynucleotide sequence at least 70 % homologous with SEQ ID NOs:9 or 13, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin, said polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

21.   The vector of claim 20, wherein said polynucleotide sequence is as set forth in SEQ ID NOs:9 or 13.

22.   A host cell comprising an exogenous polynucleotide fragment including a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity, wherein said polypeptide shares at least 70 % homology with SEQ ID NOs:10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

23.   The host cell of claim 22, wherein said polynucleotide sequence includes nucleotides 63-1691 of SEQ ID NO:9, or nucleotides 139-1869 of SEQ ID NO:13.

24.   The host cell of claim 22, wherein said polynucleotide sequence includes nucleotides 63-721 of SEQ ID NO:9.

25.   The host cell of claim 22, wherein said polynucleotide sequence is as set forth in SEQ ID NOs:9 or 13.

26.   The host cell of claim 22, wherein said polynucleotide sequence includes a segment of SEQ ID NOs:9 or 13, said segment encodes said polypeptide having said heparanase catalytic activity.

27.   The host cell of claim 22, wherein said polypeptide includes an amino acid sequence as set forth in SEQ ID NOs:10 or 14.

28.   The host cell of claim 22, wherein said polypeptide includes a segment of SEQ ID NOs:10 or 14, said segment harbors said heparanase catalytic activity.

29.   The host cell of claim 22, wherein said polynucleotide sequence is selected from the group consisting of double stranded DNA, single stranded DNA and RNA.

30.   The host cell of claim 22, wherein said cell is an insect cell.

31.   A host cell comprising a polynucleotide sequence at least 70 % homologous with SEQ ID NOs:9 or 13, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group

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(GCG) at the University of Wisconsin, said polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

32. The host cell of claim 31, wherein said polynucleotide sequence is as set forth in SEQ ID NOs:9 or 13.

33. A recombinant protein comprising a polypeptide having heparanase catalytic activity, said polypeptide shares at least 70 % homology with SEQ ID NOs:10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

34. The recombinant protein of claim 33, wherein said polypeptide includes a segment of SEQ ID NOs:10 or 14.

35. The recombinant protein of claim 33, wherein said polypeptide is as set forth in SEQ ID NOs:10 or 14.

36. An amino acid sequence as set forth in SEQ ID NOs:10 or 14.

37. An amino acid sequence homologous to SEQ ID NOs:10 or 14.

38. A pharmaceutical composition comprising, as an active ingredient, a recombinant protein including a polypeptide having heparanase catalytic activity, said polypeptide shares at least 70 % homology with SEQ ID NOs:10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

39. The pharmaceutical composition of claim 38, wherein said polypeptide includes a segment of SEQ ID NOs:10 or 14.

40. The pharmaceutical composition of claim 38, wherein said polypeptide is as set forth in SEQ ID NOs:10 or 14.

41. A modulator of heparin-binding growth factors, cellular responses to heparin-binding growth factors and cytokines, cell interaction with plasma lipoproteins, cellular susceptibility to viral, protozoa and bacterial infections or disintegration of neurodegenerative plaques comprising, as an active ingredient, a

recombinant protein including a polypeptide having heparanase catalytic activity, said polypeptide shares at least 70% homology with SEQ ID NOs: 10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the
5   University of Wisconsin.

42.   The modulator of claim 41, wherein said polypeptide includes a segment of SEQ ID NOs: 10 or 14 having heparanase catalytic activity.

43.   The modulator of claim 41, wherein said polypeptide is as set
10   forth in SEQ ID NOs: 10 or 14.

44.   A medical equipment comprising a medical device containing, as an active ingredient, a recombinant protein including a polypeptide having heparanase catalytic activity, said polypeptide shares at least 70% homology
15   with SEQ ID NOs: 10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

45.   The medical equipment of claim 44, wherein said polypeptide
20   includes a segment of SEQ ID NOs: 10 or 14.

46.   The medical equipment of claim 44, wherein said polypeptide is as set forth in SEQ ID NOs: 10 or 14.
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47.   A host cell expressing a recombinant heparanase, wherein said recombinant heparanase shares at least 70% homology with SEQ ID NOs: 10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the
30   University of Wisconsin.

48.   The host cell of claim 47, wherein said polypeptide includes a segment of SEQ ID NOs: 10 or 14.

35   49.   The host cell of claim 47, wherein said polypeptide is as set forth in SEQ ID NOs: 10 or 14.

50. The host cell of claim 47, wherein said cell is an insect cell.

51. A cell extract or conditioned cell media or a partially purified cell extract or conditioned cell media comprising an extract or media of the host cell of any of claims 22-32 and 47-50 wherein said extract or media includes a polypeptide sharing at least 70% homology with SEQ ID NOs: 10 or 14.

52. A heparanase inhibitors screening system comprising the cell extract or conditioned cell media or the partially purified cell extract or conditioned cell media of claim 51.

53. A method of screening for a heparanase inhibitor, the method comprising assaying for heparanase catalytic activity of the recombinant protein of any of claims 33-35 in the presence and in the absence of, or in the presence of varying concentrations of, at least one compound being tested for a potential at inhibiting heparanase catalytic activity.

54. A heparanase overexpression system comprising a cell overexpressing heparanase catalytic activity, wherein said heparanase catalytic activity is effected by a heparanase sharing at least 70% homology with SEQ ID NOs: 10 or 14, as determined using default parameters of a DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

55. The system of claim 54, wherein said polypeptide includes a segment of SEQ ID NOs: 10 or 14 having heparanase catalytic activity.

56. The system of claim 54, wherein said polypeptide is as set forth in SEQ ID NOs: 10 or 14.

57. A method of identifying a chromosome region harbouring a heparanase gene in a chromosome spread comprising the steps of:
    a) hybridizing the chromosome spread with a tagged polynucleotide probe at least 70% homologous with SEQ ID NOs: 9 or 13 as determined using default parameters of a DNA sequence analysis software package

developed by the Genetic Computer Group (GCG) at the University of Wisconsin;

   b)   washing the chromosome spread, thereby removing excess of non-hybridized probe; and

   c)   searching for signals associated with said hybridized tagged polynucleotide probe, wherein detected signals being indicative of a chromosome region harboring a heparanase gene.

58.   A single stranded polynucleotide fragment comprising a polynucleotide sequence complementary to at least a portion of a polynucleotide strand defined by nucleotides 226 to 721 of SEQ ID NO: 9 having heparanase catalytic activity.

59.   A polynucleotide fragment according to any one of claims 1 to 10 substantially as hereinbefore described with particular reference to the examples.

60.   A vector according to any one of claims 11 to 21 substantially as hereinbefore described with particular reference to the examples.

61.   A host cell according to any one of claims 22 to 32 or 48 to 50 substantially as hereinbefore described with particular reference to the examples.

62.   A recombinant protein according to any one of claims 33 or 34 substantially as hereinbefore described with particular reference to the examples.

63.   A pharmaceutical composition according to any one of claims 38 or 40 substantially as hereinbefore described with particular reference to the examples.

64.   A modulator according to any one of claims 41 to 43 substantially as hereinbefore described with particular reference to the examples.
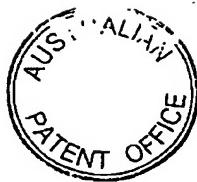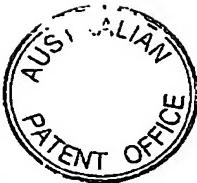
65. Medical equipment according to any one of claims 44 to 46 substantially as hereinbefore described with particular reference to the examples.

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66. A cell extract according to claim 51 substantially as hereinbefore described with particular reference to the examples.

67. An inhibitor screen system according to claim 52 substantially as hereinbefore described with particular reference to the examples.

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68. An overexpression system according to any one of claims 54 to 56 substantially as hereinbefore described with particular reference to the examples.

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69. A method according to claims 53 or 57 substantially as hereinbefore described with particular reference to the examples.

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